# SORPTION AND FUNGITOXICITY OF RADIOACTIVE POTASSIUM DIMETHYL- AND DI-N-PROPYLDITHIOCARBAMATES

DISSERTATION

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# INTRODUCTION

Fungicidal activity may be dependent upon both permeation and inherent toxicity. Since penetration of the cytoplasmic membrane may be essential for expression of toxicity, two compounds of equal inherent toxicity and water solubility, if sorbed differentially, would differ in fungicidal effectiveness.

This investigation was based on the supposition that absorption and adsorption (collectively, sorption) differences may be related to the differences in fungicidal activity of dimethyl- and di-n-propyldithiocarbamate. Bioassay and radiotracer procedures in earlier work (29) were used for orientation in the preliminary phases of this research. Since radiotracer data were quite variable (29), considerable effort was spent in the development and refinement of these procedures to obtain reliable sorption data.

Included is an investigation of certain factors affecting spore germination and fungicidal activity of the potassium salt of the dimethyl- and di-n-propyldithiocarbamates, variability in radiotracer techniques, the relationship between sorption of the homologs and their fungicidal activity, and the effect of carbon source and glucose concentration on susceptibility of fungus spores to the dimethyl derivative.

## LITERATURE REVIEW

Research on the mode of fungicidal action of the dithiocarbamates has been directed along three general lines: 1) identification of the decomposition products and evaluation of their toxicity; 2) studies on the effect of the derivatives on enzymes, particularly those of the respiratory system; and 3) investigations on the accumulation of the toxicants.

Chemical investigations on the decomposition products of the bisdithiocarbamates have revealed certain compounds which are common to the degradation of the dialkyldithiocarbamates.

Generally investigators agree that hydrolysis of disodium ethylene bisdithiocarbamate (nabam) results in the formation of carbon disulfide (15, 16, 17, 18, 31, 36). Barratt and Horsfall (3), unable to identify this gas, concluded that hydrogen sulfide was the gas evolved on hydrolysis of bisdithiocarbamate solutions. Since hydrogen sulfide may, under basic conditions, oxidize to the sulfate, this could explain why they were unable to identify this gas. Although Cox, et al., (6) concluded that the residue formed during hydrolysis was ethylenediamine, only ethylene thiourea was identified by Barratt Horsfall (3). Since carbon disulfide, hydrogen sulfide, ethylenediamine, and ethylene thiourea are not equivalent in toxicity to the parent compound, fungicidal activity of the bisdithiocarbamates may be attributed to the molecule, or ions of this group, or to an oxidative product. Barratt and Horsfall (3) postulated diisothiocyanate and hydrogen sulfide as end products in nabam

hydrolysis, but did not indicate that isothiocyanate was the fungitoxic component of nabam. According to Ludwig et al. (17), Klopping and van der Kerk presented evidence that fungicidal activity of the bisdithiocarbamates is attributed to the diisothiocyanates, which are formed during oxidation. In the residue formed during aeration, Ludwig and Thorn (16, 17) identified ethylene thiuram monosulfide (E.T.M.) its polymer and elemental sulfur. Sijpesteyn and van der Kerk, according to Ludwig (18), also identified E.T.M. in nabam solutions and suggested this compound as the source of the isothiocyanate. Recently, Ludwig, et al. (18) demonstrated that an isothiocyanate does result from E.T.M.

Hydrolysis of salts of the dialkyldithiocarbamates, (with the exception of the copper salts) results in the formation of carbon disulfide and the dialkylamine (15). Barratt and Horsfall (3), however, identified hydrogen sulfide as the only gas evolved during hydrolysis. As carbon disulfide, hydrogen sulfide and the dialkylamine are less toxic than the dialkyldithiocarbamate, fungicidal activity of this group must not be attributed to these degradation products.

According to Owens (27), Dawson and Martel, in their work with enzymes demonstrated that sodium diethyldithiocarbamate forms stable complexes with the copper atoms of copper-containing enzymes. In addition to these enzymes, Owens (27) reported further that dithiocarbamic acid derivatives inhibited sulfhydryl-, iron-, and amino-dependent enzymes; Owens and others (33) have concluded

that inhibition may result from the formation of a complex with the metals of metal-containing enzymes and by interference in electron shifts. According to Ludwig, et al. (17) Sijpesteyn and van der Kerk presented evidence that isothiocyanate reacts with the -SH groups in the cell. By the addition of metal ions, Owens (28) obtained partial reversal of the inhibitory effect of sodium diethyldithiocarbamate on polyphenol oxidase. $Ni^{++}$ and $Co^{++}$ were most effective in reversing the inhibitory action. Weed (34) reported some indication of reversal of inhibition with the addition of glutathione and cysteine on ferbam-treated spores of Neurospora sitophila.

Sorption of the toxicant in relation to toxicity is another subject of investigation on the mode of action of the dithiocarbamates. Rich and Horsfall (32), in an excellent series of investigations concerned with fungitoxicity, permeation, and lipoid solubility, found that fungicidal activity of members of homologous series of compounds (2-imidazoline and 4-nitrosopyrazole), varied depending upon the length of lipophilic side-chains. According to these authors, and in view of the results other workers obtained (1, 14), similar results were obtained by Kitajima, who reported that the toxicity of fatty acids increased with increase in number of carbon atoms from 5 to 12 beyond which it decreased. Since the toxicity of individuals of homologous series may depend upon the lipoid-water solubility ratio, this may account for the results obtained by Kitajima. The first evidence of toxicant accumulation by spores was presented by Goldsworthy in 1943 (11).

Recently, Miller, McCallan and Weed (21, 23, 24, 25), in a series of radiotracer investigations, confirmed Goldsworthy's results. In similar radiotracer work with the dialkyldithiocarbamates, Palmer (29) reported that average sorption of the more fungicidal sodium dimethyl homolog was greater than the less fungicidal sodium di-n-propyl derivative, although sorption differences were not statistically significant.

In investigations on the effect of carbon sources on fungi, Hawker (12), found that complex carbohydrates (di-, tri-, and polysaccharides) are generally less satisfactory for vegetative development and, conversely, more suitable for spore formation than the monosaccharides. Brown (4) observed that sporulation of Fusarium fructigenum decreased as glucose was increased, while mycelial development increased with a rise in glucose concentration. In addition to these results, Elliott (9) also found that spores of H. sativum increased in length as glucose concentration was reduced from 8 to 0.0625 per cent. When glucose was replaced by starch, conidial formation increased with increments of the carbohydrate up to a relatively high concentration (12). In work with Melanospora destruens (12) perithecial formation increased with increase in sucrose and starch concentrations up to 5 per cent, whereas perithecial development decreased as glucose was decreased from 2.0 to 0.5 per cent. Five per cent glucose inhibited perethecial formation.

## METHODS

### Refinement of Bioassay Techniques

In earlier work with the sodium salt of the dithiocarbamates, Palmer (29) found that average sorption of the more fungicidal dimethyldithiocarbamate was greater than that of the less fungicidal di-n-propyl homolog, although the differences in sorption were not statistically significant. Results were too variable to relate sorption to fungicidial activity. Since the reason(s) for this variability were not clear, the first phase of this research was concerned with evaluation and revision of the bioassay and radiotracer techniques used in earlier work (29).

A suitable bioassay for the test compounds must be equally amenable to radiotracer work. The fungicidal assay (2), a type of spore germination assay, seemed most appropriate for evaluating the fungicidal value of the compounds. Since the lethal dose retained in or on a spore is dependent upon the method of preparing spores for fungicidal assay, washed spores, which failed to germinate under conditions favorable to germination, are referred to in this work as dead.

To find sources of variability in the bioassay work, it was necessary to investigate and evaluate revisions against individual steps in the procedure reported earlier (29). For orientation, therefore, the following procedure was followed. Spores were removed from culture by lightly rubbing a wire loop or rubber policeman over the watercovered surface of the fungus. To free the spores from masses of mycelium and agar fragments, the

suspension was filtered through two layers of cheesecloth. Using 3-one minute centrifugations, more complete separation of spores and mycelium was obtained. Spore concentrations were estimated with the Hellige haemacytometer. Final spore concentrations of A. oleracea, H. sativum, and M. fructicola were adjusted with an aqueous solution of 500 ppm of Triton X-100 to $1x10^6$, $5x10^5$, and $1x10^6$ spores per ml, respectively. Fungicides were evaluated at $5x10^{-2}$ and $1x10^{-3}$ molar.

Five ml each of the spore suspension and fungicide solution were added to a 15 ml screw-cap vial. To reduce spore loss, the cap was lined with white gum-rubber sheeting. Duration of exposure of spores to the compound solutions was ordinarily 1 and 24 hours. Following the exposure period, the spores were separated from the fungicide solution by filtering through a modified Gooch crucible. Filtration pressure was maintained at 7.5 p.s.i. Filtration was timed to coincide with the end of the exposure period. The spores, collected on a filter paper disc (Whatman No. 50), were then rinsed to remove "extraneous" fungicide. In the bioassay work, ordinarily a single 10 ml wash was used.

Spores were removed by rinsing the surface of the filter with 3 ml of 0.1 per cent ultracentrifuged orange juice. Eight drops of the spore suspension were then pipetted onto silicone-coated glass microscope slides. (Slides were coated by exposure to the vapor of Dri-Film No. SC-77, marketed by General Electric Corporation). The slides containing the spore drops were placed in inverted Petri dishes, water-sealed to prevent evaporation,

and incubated at 20° C for 24 hours.

The number of nongerminated spores in 25 spores per drop, were counted using the 10x microscope objective. An estimate of spores killed by the fungicide was determined with Abbotts formula (13).

Selection of the Potassium Dialkyldithiocarbamates.--Compounds for study should be easily synthesized, water soluble, (equally available to fungus spores) and relatively stable at fungicidal concentrations. With reservation (precipitation of the di-n-propyl compound occurred at $1x10^{-2}$ molar within 24 hours after chemical synthesis) these were characteristic of the sodium dialkyldithiocarbamates. Although the sodium salt of the bisdithiocarbamates and the dialkyldithiocarbamates has been rather extensively investigated (3, 6, 15, 16, 17, 18, 30, 31) certain considerations favored the use of the potassium salt in this research. Davson and Danielli (7, 8) and others (20, 26) have reported that sodium has a greater effect in increasing permeability of certain plant and animal cells. Furthermore, Davson (8) in investigations with cat erythrocytes found that although penetration of $Na^+$ and $K^+$ was proportional as pH was increased from 6.0 to 7.9, permeation of sodium exceeded potassium threefold at pH 7.9. This was of particular interest as solutions of the fungicides in this work were buffered at pH 10.5, a point at which sodium penetration might be even greater. With greater permeation of $Na^+$ a greater number of oppositely charged ions,

e.g. $OH^-$ or $R\backslash R'\!>\!N\text{-}C(=S)\text{-}S^-$ may also enter unless the unbalanced electrical forces were compensated in some other manner.

To reduce the influence on permeability which Na may exert on the test spore, substitution with the potassium salt was considered desirable, provided that: 1)potassium per se and the potassium carbonate buffer pairs were relatively nonfungicidal, and 2)the potassium dimethyl and di-n-propyldithiocarbamates were similar to the respective sodium salts in fungicidal activity. Therefore, it was necessary to determine the influence of potassium and its salts on spore germination.

For comparison, both the sodium and potassium carbonate buffer systems were investigated. The chlorides of sodium and potassium were also evaluated to measure the effect of $CO_3^=$ and $HCO_3^-$ on spore germination. pH of the solutions was measured with a Beckman meter with a lithium glass electrode. Concentrations of the sodium and potassium chloride solutions were calculated to agree with the ionic strengths of their respective carbonate buffers.

Fungicidal data from this investigation are listed in Table 1. Sodium and potassium chloride had approximately the same fungicidal effect on A. oleracea spores. The two buffers were of similar toxicity.

The next step in this series of investigations was a comparison of the fungicidal activities of the potassium and sodium dialkyldithiocarbamate homologs. A summary of the effects of these salts is given in Table 2. The same relative fungicidal

differences were evident toward A. oleracea, H. sativum, and M. fructicola with both the potassium and the sodium salts.

Since the potassium buffer and dithiocarbamate salts were similar in effect to the respective sodium compounds, and in view of the reported influence of sodium on permeability of certain plant and animal cells, the potassium salts were used in subsequent work.

Selection of Medium for Harvesting Spores from Culture.--Frequently in this research, germination of A. oleracea spores was quite low. Since it was important to obtain high germination rates for accurate interpretation of sorption and fungicidal data, an investigation of the factors suspected of influencing germination was considered necessary.

One of these factors was believed associated with the method of removing spores from culture. Since conidia were obtained by rubbing the water-covered substrate surface, uniformity of the spore sample was dependent upon spore wettability and extent and pressure of rubbing the culture surface. To evaluate the effect of distilled water as a harvesting medium, a wetting agent, Triton X-100 (500 ppm) was used for comparison. The suspensions were divided equally. Spores from one of the suspensionsfrom each pair were washed with Triton. Distilled water was used to wash the spores in the two remaining suspensions.

Data from this work are listed in Table 3. Germination of spores removed from culture in Triton was markedly reduced.

Table 1.--Influence of Sodium and Potassium and Their Carbonate Buffer Systems on Spore Germination of A. oleracea.

| Treatment | Actual pH | Per cent kill |
|---|---|---|
| $Na_2CO_3$ $NaHCO_3$ | 10.25 | 14 |
| $K_2CO_3$ $KHCO_3$ | 10.15 | 7 |
| NaCl | 10.32 | 13 |
| KCl | 10.32 | 11 |

Table 2.--Comparison of Fungicidal Activity of Sodium and Potassium Dimethyl- and Di-n-propyldithiocarbamates.

| Derivative | Concentration (molar) | Per cent kill | | |
|---|---|---|---|---|
| | | A. oleracea | H. sativum | M. fructicola |
| Sodium dimethyl | $5x10^{-3}$ | -- | 100 | 100 |
| | $1x10^{-3}$ | 84 | 50 | 100 |
| Sodium di-n-propyl | $5x10^{-3}$ | -- | 5 | 11 |
| | $1x10^{-3}$ | 46 | 8 | 4 |
| Potassium dimethyl | $5x10^{-3}$ | -- | 100 | 100 |
| | $1x10^{-3}$ | 75 | 36 | 83 |
| Potassium di-n-propyl | $5x10^{-3}$ | -- | 0 | 20 |
| | $1x10^{-3}$ | 24 | 0 | 4 |

Table 3.--Influence of Harvesting Medium on Germination of A. oleracea spores.

| Spore wash | Average per cent kill in two tests | |
|---|---|---|
| | Harvesting Medium | |
| | Triton X-100 | Distilled water |
| Distilled water | 66 | 91 |
| Triton X-100 | 55 | 82 |

Reduced germination was also evident with spores obtained from culture in distilled water and washed with Triton. Although removing

conidia from culture in distilled water had little deleterious effect on spore germination in these particular tests, a more suitable method for collecting conidia was desired.

This was based on the supposition that materials on the substrate may influence the fungicidal effect of the dimethyl derivatives. These substances, if sorbed by the spores, might precipitate the derivatives or inactivate them by adsorption. Such an effect could alter the sorption-toxicity ratio. Consequently, the fungicidal effect of the dimethyl compound was examined against conidia harvested dry.

To evaluate this effect spores were harvested from culture with deionized water* (wet method) and by vacuum (dry method), using a modified cyclone separator. Following spore washes, both groups of spores were finally suspended in an aqueous solution of 500 ppm of Triton X-100. All treatments evaluated with the dimethyl compound at $1x10^{-3}$ molar, were replicated twice.

Results from this work are presented in Table 4. A greater percentage of spores removed from culture by the dry method were killed by the dimethyl homolog. The interesting point in this work was the relative ineffectiveness of spore rinses to remove the material or materials that apparently inactivate the fungicide. Since these substances could influence retention or actual sorption in or on the spores, conidia in subsequent work were removed from

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* Deionized water was prepared by eluting distilled water through a column containing a cation-anion exchange resin (Amberlite MB-2, Fisher Chemical Company)

Table 4.--Effect of the Method of Harvesting Spores from Culture on the Fungicidal Effectiveness of the Dimethyl Derivative ($1 \times 10^{-3}$ molar).

| Fungus species | Average per cent kill in two tests<br>Method of spore removal from culture<br>Wet | Dry |
|---|---|---|
| A. oleracea | 10 | 80 |
| H. sativum | 18 | 64 |

| Comparison | Test | Data | 5% | 1% | Significance |
|---|---|---|---|---|---|
| A. oleracea (wet vs. dry) | t | 35.0 | 4.3 | 9.9 | 1% |
| H. sativum (wet vs. dry) | t | 23.0 | 4.3 | 9.9 | 1% |

culture with vacuum.

Although satisfactory germination rates of H. sativum spores were obtained with the vacuum method, germination of A. oleracea spores remained quite low and use of the fungus was discontinued.

Desorption of the Dimethyl and Di-n-propyl Derivatives.--Although the chemical properties of the dimethyl and di-n-propyl derivatives are closely similar, the physical characteristics of the di-n-propyl compound may be markedly influenced by the additional $CH_2$ groups. Thus even though sorption of this derivative was similar to the dimethyl homolog, the compound might be retained less strongly in or on the spore. Since a 45 ml wash volume was used in earlier work (29) to remove extraneous fungicide, it was considered that such a wash volume might desorb unequal amounts of the compounds. This possibility was of particular importance in radiotracer work.

Since it was not possible at this point to evaluate the effect of wash volumes on desorption, per cent kill was selected as an index of the effect of spore rinses on desorption of the derivative. Three rinse volumes were tested; 2, 10, and 45 ml. After filtration of the spore-fungicide suspension to the 1 ml level, the vial was washed with 2 ml of distilled water, and the rinsing emptied into the filter unit. The remaining volumes of the 10 ml and 45 ml rinses were added in portions of 3 and 5 ml.

Data from this work are listed in Table 5. As would be expected maximum kill resulted when spores were washed with the least rinse volume. Germination of M. fructicola spores was proportional to volume of wash, while the 10 and 45 ml washes desorbed approximately the same amount of the dimethyl derivative from H. sativum spores. Since the di-n-propyl homolog was relatively non-toxic it was not possible to evaluate the effect of rinses on di-n-propyl-treated spores. As it was not possible at this point to determine quantitative desorption with radiotracer compounds, the 10 ml wash volume was selected for use in subsequent research. This was the smallest volume that could be used with a minimum spore loss (approximately 1 per cent).

## Refinement of Radiotracer Procedures

The relationship between sorption and fungicidal activity of the dialkyldithiocarbamates, was investigated using the $S^{35}$ - labelled dimethyl and di-n-propyl derivatives. As earlier results on sorption of the sodium derivatives were too variable for correlation

(29), two possibilities appeared evident at this point: 1) modify existing techniques, and, more basically 2) locate the site or sites of sorption by autoradiographic analysis of spore sections. Although the first of these was selected, preliminary work on the latter was started.

For orientation in the preliminary phases of the sorption work, certain procedures used earlier were followed (29). Five ml of the $S^{35}$ - labelled fungicide solution were added to vials containing an equal volume of spore suspension. The vials, capped with gum rubber-lined lids, were rotated end over end during the exposure period. Controls (distilled water substituted for the spore suspension) were run with each compound. The spores were separated from the fungicide solution by filtration. The filtered spores and control filter discs were then washed with nine successive 5-ml portions of distilled water. One ml was retained in the filter unit between each wash. The end of filtration was timed to coincide with the end of the exposure period. After drying, the filter disc was measured for beta activity with a Geiger tube and linear scaler. Radioactivity was determined over a 20-minute period and recorded in counts per minute (C/M).

Fungicidal activity was determined concurrently under conditions similar to the radiotracer work.

Retention of the Dimethyl Compound by the Filter Disc.--Extensive variability in the radioactivity of the replicate filter discs in earlier work (29) could be attributed to the filter unit per se, to

spore loss during filtration, or to absence of a spore monolayer on the cellulose filter. The filter assembly as the source of variability was favored for several reasons. First of all, with the low radioactivity per spore indicated in previous work (29), a loss of nearly all spores would be necessary to account for replicate variation. Second, that a monolayer of spores was obtained on the cellulosic filter (Whatman No. 50) was verified by microscopic examination. Third, replicate variation of the spore sample was similar to the control filter units. Therefore, it was evident that variability should be associated with the filter disc unit.

Differences in retention of the radioactive compounds in or on the cellulose filter may cause this variability in activity of the control filter disc. To determine the influence of the paper filters on fungicide retention, 3 stainless steel frits were selected at random and fitted with cellulose paper circles. Ten ml of the radioactive dimethyl derivative were then filtered through each paper disc using a pressure of 7.5 p.s.i. The filters were drained for one minute under vacuum after the disappearance of free liquid, and then dried in a vacuum desiccator. The disc was measured for radioactivity. The test was repeated after the stainless steel unit was washed thoroughly and checked for radioactivity above background. Five such filtrations were made with the same filter disc. Radioactivity is indicated in counts per minute (C/M).

Data are presented in Table 6. Marked differences in activity occurred among filter paper circles with the same frit, and, as would be expected, between frits. Therefore, such differences in retentiveness by the cellulose filters could mask moderate sorption differences by the spores.

Relation of Filtration Pressure and Fungicide Retention.--Since filtration pressure was sometimes difficult to control, this variability may also influence the amount of compound retained by the filter. To determine whether retention of the compound by the filter was correlated with filtration pressure, radioactive fungicide solutions were filtered through the unit at various pressures.

Results obtained are listed in Table 7. Variability per se is not related to filtration pressure and is not, therefore, a function of fungicide retention.

Effect of Pre-Treatment of the Filter Paper on Retention.--Since non cellulosic materials in the filter paper may precipitate the dialkyldithiocarbamates and thus contribute to variability, attempts were made to free these substances from the filter. Filter paper (Whatman No. 50) circles were boiled for 5 minutes in a 0.5 normal ammonium chloride solution (prepared by mixing 0.5 normal HCl with an equal volume of an equinormal solution of ammonium hydroxide). The hot liquid was decanted and the filter circles transferred to a clean beaker. Deionized water was added, boiled for 5 minutes, and the liquid removed. This was repeated until the test for ammonia in the filters was negative. Usually only three washings

Table 6.--Retention of the Dimethyl Compound by the Cellulose Filter.

| Replicate number | Radioactivity as based on C/M Filter disc number 10 | 12 | 23 |
|---|---|---|---|
| 1 | 101 | 62 | 77 |
| 2 | 99 | 84 | 283 |
| 3 | 75 | 246 | 119 |
| 4 | 54 | 331 | 252 |
| 5 | 309 | 274 | 294 |

Table 7.--Effect of Filtration Pressure on Retention of the Dimethyl Compound.

| Filter disc | Retention of the treated filter discs based on C/M above background | Filtration pressure (p.s.i.) |
|---|---|---|
| 20 | 210 | 15 |
| | 291 | 20 |
| | 272 | 40 |
| 4 | 320 | 15 |
| | 295 | 20 |
| | 268 | 40 |

in boiling deionized water were necessary to leach the ammonia from the filters. Controls were prepared concurrently substituting deionized water. After treatment, the cellulose circles were immersed in deionized water until ready for use. To evaluate the influence of the treatments, a single filter frit was used to filter the radioactive dimethyl compound. Radioactivity is expressed in counts per minute (C/M).

Data from this work are listed in Table 8. The ammonium chloride treatment considerably reduced variation in retention of the fungicide by the filter paper discs. Although variation was

Table 8.--Retention of the Dimethyl Derivative by Filter Paper Treated with Ammonium Chloride.

| Filter paper treatment | Retention of the treated filter discs based on C/M above controls |
|---|---|
| Ammonium chloride | 173 |
| | 102 |
| | 97 |
| | 100 |
| Deionized water | 407 |
| | 230 |
| | 95 |
| | 221 |

satisfactorily reduced by this treatment, pore size of the cellulose filter was so greatly enlarged, that M. fructicola spores and smaller spores of H. sativum were not separated by the filter from suspension. In subsequent work, spores were collected on plastic "Millipore" filters (Lovell Chemical Company).

Other Sources of Variability.--In earlier work, lids of the screw-cap vials were lined with white gum-rubber sheeting to reduce spore loss during agitation of the spore-fungicide suspension. Although these liners and the addition of the surface active agent reduced spores loss to the 1 per cent level (29), occasionally spores of H. sativum adhered to the gum-rubber liner. These spores, which were extremely difficult to remove by ordinary rinsing procedures, obviously were not adequately exposed to the toxicant during the exposure period. Since none of the liners tested were satisfactory, screw caps in later radiotracer work were not lined.

Another source of variability resulted from the loss of spores between the filter disc and the main filter unit. With a pressure of approximately 7.5 p.s.i. during filtration, the surface of the filter fitted tightly against the filter cone. When pressure released at the end of filtration or between additions of rinse volumes, liquid would occasionally seep between the cone and disc. This resulted in clumping of the spores at the edge of the filter. Since this would result in reduced measurable radioactivity, clumping could result in serious error. To correct this, a new filter unit was designed and built (Fig. 1).

The assembly consisted of two parts: the filter housing and the filter disc. In the filter disc recess at the base of the housing unit a rubber O-ring was inserted to retain the disc tightly against this housing unit during filtration. The disc, fitted with a coarse ceramic filter (Kauffman and Lattimer) was covered with a cellulose filter (Whatman No. 50) circle. The plastic filter was then placed on the upper surface of the cellulose paper. This prevented rupture of the delicate plastic membrane by the ceramic filter.

It was considered advisable before proceeding with sorption studies to determine the effectiveness of the filter unit with respect to spore distribution and relative number of spores on the plastic filter. In addition, it was of interest to know whether significant differences in number and distribution of spores occurred between aliquots of the same and separately prepared spore suspensions.

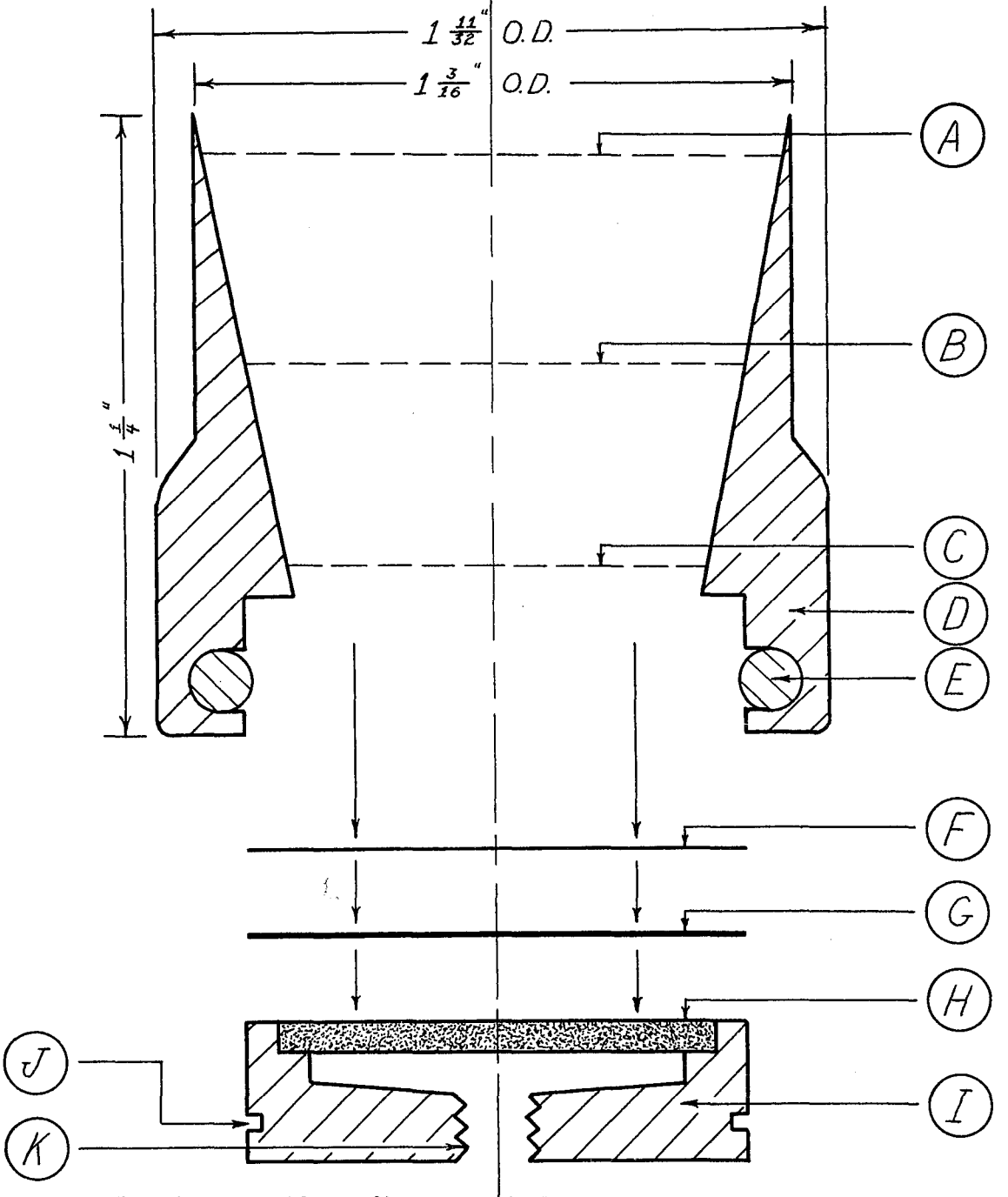


Figure 1. Diagrammatic Median Longitudinal Section of Filter Unit

A- 10 ml level
B- 6 ml level
C- 1 ml level
D- Stainless steel filter cone
E- Rubber "O" ring
F- Micropore filter
G- Cellulose filter
H- Ceramic filter
I- Stainless steel filter disc
J- "O" ring groove
K- Threaded outlet

Particular care was used in removing aliquots from the stock spore suspension to obtain representative samples of spores. Spore suspensions were placed in 100 ml graduate cylinders, and inverted three times. The tip of a 5 ml volumetric pipette was then immediately immersed 10 ml below the surface of the suspension, and the sample removed within 15 to 20 seconds.

Five ml of the spore suspension were added to an equal volume of the fungicide solution contained in a 15 ml screw-cap vial. The plastic cap was unlined. Following the spore-fungicide contact period, the suspension was filtered at 2 p.s.i. The plastic filters were removed from the filter disc and mounted on a glass microscope slide.

The filter disc was divided into 3 equal areas. Each area was sampled by selecting 15 random fields using the 10x objective of the circular stage microscope.

Data from the samplings are given in Table 9. The letters A and B of the sample number refer to different master spore suspensions, while the number indicates the replicate. Differences in spore number and spore distribution between areas and between tests, using the same or a separately prepared spore suspensions, are not significant. Of the 315 fields examined clumping of spores was never observed.

Table 9.--Spore Distribution on Plastic Filter.

| Sample number | Derivative | Number of spores per area A | B | C | Total |
|---|---|---|---|---|---|
| A-1 | Dimethyl | 545 | 604 | 568 | 1717 |
| A-2 | do | 647 | 513 | 556 | 1716 |
| A-3 | do | 531 | 569 | 566 | 1666 |
| B-1 | do | 576 | 557 | 504 | 1637 |
| A-1 | Di-n-propyl | 516 | 639 | 782 | 1937 |
| A-2 | do | 537 | 596 | 687 | 1820 |
| B-2 | do | 556 | 468 | 373 | 1397 |

| Source | D.f. | Ss | Ms. | F | F.01 |
|---|---|---|---|---|---|
| Treatment | 6 | 3,734.1 | 622.4 | 1.45 | 4.82 |
| Area | 2 | 82.3 | 41.1 | 0.10 | 6.93 |
| Interaction | 12 | 5,146.1 | 428.8 | 1.65 | 2.25 |
| Error | 294 | 76,547.2 | 260.4 | | |

## Techniques Adopted

At this stage in the development and modifications of methods, it was believed that variability had been sufficiently investigated to revise the bioassay and radiotracer procedures. Details of this phase are as follows: Fungus spores were removed from culture with vacuum and suspended in deionized water. The suspension was then filtered through two layers of cheesecloth and the spores further separated from mycelial fragments by 3-one minute centrifugations. The final spore suspensions of H. sativum and M. fructicola were adjusted with 500 ppm aqueous solutions of Triton X-100, to 50,000 and 100,000 spores per ml, respectively. At intervals of 10 minutes, 5 ml of the spore suspension were pipetted into a 15 ml screw-cap vial containing an equal amount of the fungicide solution. The vial was then rotated end on end during

the exposure period. Spores of M. fructicola and H. sativum were exposed for 1 and 24 hours, respectively. Treatments in the sorption and bioassay tests were randomized to reduce variability.

Following the contact period, 9 ml of the suspension were filtered under a tension of 2 p.s.i. The unlined vial cap and vial were then rinsed with 2 ml of deionized water and the rinsings added to the filter unit. After adding an additional 3 ml, the suspension was again withdrawn to the 1 ml level. The inside of the unit was then washed with an additional 5 ml. Final filtration was standardized by extending filtration 1 minute beyond the disappearance of free liquid from the filter surface. The plastic filter was then removed and dried in a covered Petri dish. Beta activity, measured in counts per minute (C/M), was estimated with a Geiger tube and linear scaler. Twenty-minute counting periods were used. Radioactivity values representing the extent of sorption in or on the treated spores were obtained by subtracting the radioactivity of the appropriate control filter from the radioactivity of the filter bearing the treated spores.

In the bioassay work, spores were removed from the filter with 1 ml of deionized water and the resulting suspension adjusted with an equal volume of 1 per cent ultracentrifuged orange juice. Drops of the spore suspension, pipetted onto silicone-treated slides, were placed in water-sealed Petri dishes and incubated at 20° C. After 20-24 hours, per cent spore germination was calculated.

## Autoradiography of Spore Sections

Since site of fungicidal action of the dialkyldithiocarbamates probably occurs within the spore, it was important to distinguish external and internal sorption of the compounds.

Several approaches were considered: (1) fractionation of the spore and measurement of radioactivity of the fragments; and (2) autoradiography of spore sections. To determine spore wall adsorption and absorption in the protoplasm by fractionating spores, would be extremely difficult. Since precise measurement of sorption by the spore wall and protoplasm is dependent on complete and separate recovery of the fragments, or recovery of representative samples of known amount of each component, this approach could result in considerable error. In addition to these sources of error, separation of the debris within a liquid medium, could result in uneven desorption of the compounds from the fragments. Therefore, a more direct and less severe method was considered necessary for differentiating between sorption in or on the spore wall and in the protoplasm.

Autoradiography appeared admirably suited for this work. With this technique, location of the radioactive compounds could be determined in situ. The degree of resolution required to identify sites of beta emission from intracellular structure probably would be of the order of a few microns. One of the factors necessary to obtain such definition is the use of an isotope with a relatively short range of radiation (35). Weak beta emitters such as $C^{14}$

and $S^{35}$ have a respective maximum range of radiation of 90 and 100 microns (10). Therefore, exposure of sections of $S^{35}$ - labelled cells to nuclear track emulsions could result in an image too diffuse to locate the source of radioactivity. This disadvantage may be offset by reducing exposure time and lengthening the period of emulsion development, a technique that Campbell (5) found successful. Although tritium, a hydrogen isotope with a very short range of radiation, was more applicable than $S^{35}$ to this problem, chemical procedures for the substitution of this element into the dialkyldithiocarbamate molecule were not available. Therefore, the $S^{35}$ - labelled fungicides were used in preliminary work.

During preparation of the spores for autoradiography, it was necessary to avoid, or at least minimize, desorption of the radioactive fungicides. Furthermore, it was important to avoid the exposure of radioactive materials other than the treated spores to the photographic emulsion. Details of the procedure were as follows: after conventional filtration of the radioactive solution, the spores were removed from the filter. This was accomplished by coating another nonradioactive plastic filter with 1 per cent gelatin and pressing the spore-gelatin surfaces firmly together. This usually resulted in removal of at least 50 per cent of the spores from the filter. To prevent loss of spores and to reduce leaching of the fungicide from the spores during preparation for imbedding, the filter surface was coated with a thin film of 1 per cent gelatin. After air-drying for approximately 12 hours, the

filter was cut into one-quarter-inch squares which were immersed in the fixative, a solution of alcohol, formalin, and acetic acid (100:1:1). After 48 hours, the specimens were dehydrated by immersing them in increasing concentrations of ethyl alcohol (30, 50, 70, 80, 95, and 100 per cent). Prior to imbedding in paraffin, spores were infiltrated with benzene. Sections 5 microns thick were cut with the sliding microtome and affixed to glass microscope slides coated with albumin. To locate the sources of radioactivity, track autoradiography (paths of the radioactive particles) was considered most desirable. Liquid $NTB_3$ emulsion (Eastman Kodak) was applied to the mounted sections with a medicine dropper. Two drops of the emulsion applied per square inch resulted in a thickness of about 5 microns. The emulsion, which in the liquid form is insensitive to background radioactivity, was air-dried at room temperature. The sections were exposed to the emulsion in light-proof containers at $5^{\circ}$ C for 8, 12, and 24 hours. The autoradiographs were developed with amidol-sodium sulfite and fixed with F-24 (Eastman Kodak).*

At magnifications of 645, there was no evidence in the emulsion of beta emissions from the spore section, although the presence of randomly distributed reduced silver grains, indicated that the emulsion was sensitive to background radioactivity. The absence of radioactivity from the spore section may be attributed to poor contact between the emulsion and the specimen.

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* R. Doig, of Battelle Memorial Institute prepared the autoradiographs.

## Antecedent Effect of Carbon Source on Susceptibility to Dimethyldithiocarbamate

Since complex carbon sources, in general, are more suitable for spore formation than monosaccharides, it was of interest to investigate the influences of these sources on susceptibility to the dimethyl compound.

Glucose, galactose, sucrose, lactose, and starch were used as sources of carbon. The fungus, H. sativum, was cultured on basal media consisting of 2 gm $NaNO_3$, 1 gm $KH_2PO_4$, 20 g carbon source, 0.2 mg of $Fe^{+++}$, and $Zn^{++}$, 0.1 mg of $Mn^{++}$, and 20 g agar per liter of medium. The medium was adjusted to pH 6.0 with 6 normal NaOH, and autoclaved.

The influence of carbon source on susceptibility was measured by fungistatic effect (2). Dilutions of the compound were prepared with 500 ppm of an aqueous solution of Triton X-100. Tests were run in duplicate.

Glucose Concentrations.--The next phase of work on the investigation of antecedent effects of carbon sources was concerned with evaluating the influence of carbohydrate concentration on susceptibility. Glucose was selected as the source of carbon because: 1) glucose is a common source for the culture of fungi, and 2) it was of interest to determine the relationship of morphological variation (4) and susceptibility to the dimethyl compound.

The procedure for evaluating the effect of glucose concentration on susceptibility was the same as discussed under carbon

sources. Concentrations of the monosaccharide were varied between 0.125 and 4.0 per cent. Tests were repeated twice.

## RESULTS AND CONCLUSIONS

### Radiotracer Investigations

In this work the sorption-toxicant relationship was evaluated with spores of H. sativum and M. fructicola. Since spore germination rates of A. oleracea were not satisfactory, it was considered advisable to discontinue the use of this fungus. The compounds, used at a final concentration of $1x10^{-3}$ molar, were prepared directly from dilute solution and buffered with the carbonate buffer system. Ordinarily two replicates were used for each treatment in radiotracer and bioassay work. Since it was necessary to increase the number of radiotracer treatments in certain experiments, bioassay work in these tests was limited to a spore germination assay to check spore viability.

Effect of the Radioactive Compounds on the Test Fungi.--Since it was advisable to determine the influence of radioactivity at the level used in sorption work, the fungicidal effect of radioactive and nonradioactive solutions of the dialkydithiocarbamates were compared.

Data from this initial test are presented in Table 10. Fungicidal activity of radioactive and nonradioactive compounds were similar for both fungus species. Furthermore, the fungicidal difference between dimethyl and di-n-propyl homologs is also apparent with the tracer compounds.

Sorption of Dimethyl- and Di-n-propyldithiocarbamate.--Since radioactivity, as measured by fungicidal effect, did not influence sensitivity of the spores to the compounds, an investigation of the

Table 10.--Fungicidal Effects of the Radioactive and Nonradioactive Dimethyl and Di-n-propyl Homologs.

| Fungus species | Derivative | Average per cent kill in two tests: Radioactive treatment | Nonradioactive treatment |
|---|---|---|---|
| H. sativum | Dimethyl | 75 | 73 |
| | Di-n-propyl | 5 | 7 |
| M. fructicola | Dimethyl | 96 | 100 |
| | Di-n-propyl | 39 | 40 |

relationship of sorption and fungitoxicity was now justified. Results from this test are listed in Table 11. Sorption of the di-n-propyl compound was greater than the more fungicidal dimethyl derivative. Although the sorption differential occurred with both fungus species, the magnitude of the difference was less with M. fructicola, as also were differences in fungicidal activity.

Desorption of the Dimethyl and Di-n-propyl Compounds.--Although sorption appeared inversely related to fungicidal activity, it had not been conclusively demonstrated that sorption differences were real. For example, sorption of the derivatives may be similar, but, assuming retention is proportional, within limits, to aliphatic chain length, the dimethyl compound could have been desorbed more readily by filtration. Therefore, it was necessary to determine whether the compounds were desorbed differentially. To ascertain the effect of the rinse on desorption, the spore-fungicide suspension was transferred directly to the unit and the spores filtered without washing. In conjunction with this work, a similar test using the routine 10 ml spore rinse was run. In this work, the bioassay was

Table 11.--Sorption and Fungicidal Effect of Potassium Dimethyl- and Di-n-propyldithiocarbamates.

| Fungus species | Derivative | Replication | Sorption based on C/M above control | Fungicidal effect based on per cent kill |
|---|---|---|---|---|
| H. sativum | Dimethyl | 1 | 13 | 75 |
| | | 2 | 23 | |
| | | Av. | 18 | |
| | Di-n-propyl | 1 | 157 | 5 |
| | | 2 | 147 | |
| | | Av. | 152 | |
| M. fructicola | Dimethyl | 1 | 18 | 96 |
| | | 2 | 23 | |
| | | Av. | 21 | |
| | Di-n-propyl | 1 | 65 | 39 |
| | | 2 | 68 | |
| | | Av. | 67 | |

| Comparison | Test | Data | 5% | 1% | Significance |
|---|---|---|---|---|---|
| H. sativum (methyl vs. propyl) | t | 19.1 | 4.3 | 9.9 | 1% |
| M. fructicola (methyl vs. propyl) | t | 19.3 | 4.3 | 9.9 | 1% |

limited to a spore viability test, to permit a greater number of radiotracer treatments.

Results from this work are presented in Table 12. Not only was less dimethyl sorbed by spores, but a much greater proportion of that sorbed was removed by the washes; what is held, is loosely held. Since the same sorption relationship also occurred with the respective controls (plastic filters), apparently the adsorptive properties of the di-n-propyl compound are markedly different from the dimethyl homolog.

Table 12.--Desorption of Dimethyl- and Di-n-propyldithiocarbamate from Spores of H. sativum.

| Derivative | Retention by sample based on C/M above background | | Sorption by spores based on C/M | |
|---|---|---|---|---|
| | Unwashed | 10 ml wash | Unwashed | 10 ml wash |
| Dimethyl | 230 | 42 | 43 | 17 |
| Dimethyl control | 187 | 25 | | |
| Di-n-propyl | 545 | 361 | 220 | 131 |
| Di-n-propyl control | 325 | 230 | | |

Sorption of the Compounds by Dead Spores.--As the method used in radiotracer work for measuring sorption was based on radioactivity of the spore sample, the liberation of $S^{35}$ in the form of gaseous sulfur compounds, such as carbon disulfide and hydrogen sulfide, could not be measured. Since fungus spores may reduce sulfur to hydrogen sulfide (3, 13, 19, 22) this could account for the low level of radioactivity of the dimethyl treated-spores. Therefore, it was necessary to determine the influence of the biological system on sorption.

One group of spores was killed before exposure to the compounds by heating the spore suspension to 100° C for one minute (dead group). The nonheat-treated group served as the control (untreated group). Data from this work are listed in Table 13. Difference in sorption of the dimethyl compound by the dead and untreated groups, was not significant. In comparison, sorption of the di-n-propyl homolog was markedly different between the two groups of spores tested. These data are evidence that sorption differences can not be attributed to the biological system alone.

Table 13.--Sorption of Dimethyl- and Di-n-propyldithiocarbamate by Heat-treated Spores of H. sativum.

| Derivative | Replication | Sorption by spores based on C/M | | Fungicidal activity based on per cent kill |
|---|---|---|---|---|
| | | Pre-killed | Untreated | |
| Dimethyl | 1 | 16 | 15 | 77 |
| | 2 | 19 | 19 | |
| | Av. | 18 | 17 | |
| Di-n-propyl | 1 | 187 | 147 | 3 |
| | 2 | 180 | 144 | |
| | Av. | 184 | 146 | |

Effect of Exposure Duration on Sorption and Fungicidal Activity.--The final investigation in this series of tests was based on the relationship of time to sorption and fungicidal activity. Since, in previous work, spores of H. sativum were exposed to the compounds for 24 hours, the effect of the dimethyl compound on retention of the derivative was not known. Thus large amounts may be sorbed initially by the spores, but rapidly extruded as death occurs. In addition, it was also of interest to determine whether sorption of the derivatives was similar during the early part of the exposure period.

In this test, spores were exposed for intervals of five minutes, one hour, and 24 hours. Since 4 to 4.5 minutes were necessary to complete filtration procedures, exposure periods less than 5 minutes were not possible.

Data obtained from this work are given in Table 14. Sorption and fungicidal activity of the dimethyl homolog increased during the period studied. The sorption differential between the

Table 14.--Effect of Various Exposure Periods on Sorption and Fungicidal Activity of Potassium Dimethyl- and Di-n-propyldithiocarbamate ($1x10^{-3}$ molar) Using Conidia of H. sativum.

| Derivative | Exposure period duration | Sorption by conidia based on C/M | Fungicidal activity based on per cent kill |
|---|---|---|---|
| Dimethyl | 5 min. | 8 | 12 |
| Di-n-propyl | | 52 | 8 |
| Dimethyl | 1 hr. | 13 | 30 |
| Di-n-propyl | | 102 | 7 |
| Dimethyl | 24 hrs. | 29 | 52 |
| Di-n-propyl | | 265 | 9 |

| Comparison | Test | Data | 5% | 1% | Significance |
|---|---|---|---|---|---|
| Dimethyl vs. Di-n-propyl (5 minutes) | t | 20.5 | 4.303 | 9.925 | 1% |
| Dimethyl vs. Di-n-propyl (1 hour) | t | 39.01 | 4.303 | 9.925 | 1% |
| Dimethyl vs. Di-n-propyl (24 hours) | t | 135.01 | 4.303 | 9.925 | 1% |

dimethyl and di-n-propyl compounds, evident within the first five minutes, increased slightly throughout the exposure period.

From these data, there is no evidence that large amounts of the dimethyl compound are sorbed and then extruded by the spores.

## Antecedent Effect of Various Carbohydrates on Susceptibility of H. sativum to Dimethyldithiocarbamate

Differences in degree of sporulation and morphological characteristics were associated with the carbon sources. A summary of these data are listed in Table 15.

Sporulation of H. sativum on the starch medium was markedly greater than on other carbon sources. Although the number of mono-,

Table 15.--Effect of Carbon Source on Sporulation, Spore Length, Width, and Number of Cells (H. sativum).

| Carbon source | Sporulation per slant (1000 spores) | Average of 25 spores Length (μ) | Width (μ) | Number of cells |
|---|---|---|---|---|
| Glucose | 3,340 | 58.62 | 23.8 | 4.5 |
| Galactose | 1,840 | 75.41 | 26.52 | 6.6 |
| Sucrose | 3,740 | 62.56 | 22.98 | 5.0 |
| Lactose | 3,040 | 71.06 | 25.70 | 5.7 |
| Maltose | 2,510 | 59.02 | 23.46 | 5.0 |
| Starch | 11,235 | 73.61 | 28.35 | 7.2 |

| Variable | L.S.D. .01 | L.S.D. .05 |
|---|---|---|
| Length | 0.689 | 0.520 |
| Width | 0.287 | 0.216 |
| Number of cells | 0.741 | 0.560 |
| Sporulation | 3922.000 | 2501.000 |

di-, and polysaccharides tested was limited, increased sporulation was not related to the complexity of the carbohydrate. Of the di- and polysacoharides, sucrose was the most favorable for sporulation. Spores harvested from the glucose medium were significantly shorter and contained significantly fewer cells per spore, than those from other carbon sources. Greatest spore length was obtained on the galactose medium, the poorest substrate for sporulation, while significantly greater spore width and number of cells per spore resulted on the starch medium. Cell numbers and spore width are related, but not proportionally to spore length. The largest values obtained for these characters were found with galactose, lactose and starch, mone-, di-, and polysaccharide respectively. One of the most interesting observations from this work was the uniformity in size of the spores harvested from the starch medium.

Effect of Carbon Source on Susceptibility.--Data from this work are listed in Table 16. Differences in susceptibility to the dimethyl homolog were evident at 2 x $10^{-4}$ molar; spores obtained from media containing galactose, lactose, and starch were the most resistant. These spores were harvested by rinsing culture with deionized water. Such spores were longer, broader and contained a greater number of cells per spore. The dimethyl derivative was least effective against spores from the galactose medium. At concentrations of 1 x $10^{-4}$ molar and below, spores from the glucose medium were, in general, most susceptible. Under the conditions of this test, susceptibility of H. sativum to the dimethyl derivative was influenced by the carbon source.

The next phase of work on the investigation of antecedent effect of carbon sources, was concerned with glucose concentrations and their effect on susceptibility of H. sativum to dimethyldithiocarbamate.

Glucose concentrations, which were varied between 0.125 and 4.0 per cent, influenced sporulation, spore size, and number of cells per spore. A summation of the measurements of these characters are listed in Table 17.

Decreasing dextrose concentration from 4 to 1 per cent resulted in a threefold increase in sporulation. With concentrations below 1 per cent, sporulation decreased sharply. Differences in length, and number of cells per spore harvested from media containing from 0.125 to 2.0 per cent glucose, were significantly greater than media with 4 per cent glucose. Although spore length

Table 16.--Effect of Carbon Source in Culture Medium on Inhibition of *H. sativum* by Dimethyldithiocarbamate.

| Carbon source | Per cent inhibition at indicated molar concentrations | | | | |
|---|---|---|---|---|---|
| | $2x10^{-4}$ | $1x10^{-4}$ | $5x10^{-5}$ | $2.5x10^{-5}$ | $1.25x10^{-5}$ |
| Glucose | 96 | 67 | 56 | 37 | 28 |
| Galactose | 60 | 51 | 43 | 30 | 15 |
| Sucrose | 100 | 67 | 37 | 13 | 0 |
| Lactose | 43 | 36 | 24 | 16 | 9 |
| Maltose | 94 | 57 | 44 | 34 | 21 |
| Starch | 61 | 47 | 35 | 22 | 12 |

Table 17.--Effect of Glucose Concentration on Sporulation, Spore Length, Width, and Number of Cells (*H. sativum*).

| Per cent glucose concentration | Sporulation per slant (1000 spores) | Average of 25 spores | | |
|---|---|---|---|---|
| | | Length (μ) | Width (μ) | Number of cells |
| 4 | 1230 | 54.26 | 30.32 | 3.7 |
| 2 | 2210 | 62.73 | 32.13 | 5.4 |
| 1 | 3920 | 67.66 | 32.98 | 6.2 |
| 0.5 | 2132 | 74.48 | 33.32 | 5.5 |
| 0.25 | 750 | 70.72 | 26.18 | 5.1 |
| 0.125 | 465 | 72.90 | 29.41 | 6.1 |

| Variable | L.S.D.$_{.01}$ | L.S.D.$_{.05}$ |
|---|---|---|
| Length | 0.517 | 0.392 |
| Width | 0.208 | 0.157 |
| Cells per spore | 0.237 | 0.180 |
| Sporulation | 3797. | 2396. |

and width increased with decreasing glucose concentration down to 0.5 per cent, a secondary increase occurred at 0.125 per cent. One per cent glucose was optimum for maximum number of cells per spore. Here again a secondary increase is evident at 0.125 per cent.

Effect of Glucose Concentrations on Susceptibility.--The procedure for evaluating the effect of glucose concentration on susceptibility was the same as discussed under carbon sources. These data are given in Table 18. Differences in susceptibility are not evident. Apparently glucose concentrations do not influence susceptibility to the dimethyl compound.

Table 18.--Effect of Glucose Concentration in Culture Medium on Inhibition of H. sativum by Dimethyldithiocarbamates.

| Per cent concentration of glucose | Per cent inhibition at indicated molar concentrations | | | | |
|---|---|---|---|---|---|
| | $2x10^{-4}$ | $1x10^{-4}$ | $5x10^{-5}$ | $2.5x10^{-5}$ | $1.25x10^{-5}$ |
| 4 | 85 | 73 | 55 | 41 | 30 |
| 2 | 91 | 75 | 60 | 40 | 21 |
| 1 | 86 | 69 | 54 | 35 | 18 |
| 0.5 | 98 | 82 | 65 | 47 | 25 |
| 0.25 | 97 | 83 | 61 | 41 | 28 |
| 0.125 | 95 | 81 | 57 | 59 | 22 |

## DISCUSSION

$S^{35}$ - labelled potassium dimethyldithiocarbamate was more fungicidal than the corresponding di-n-propyl compound to spores of M. fructicola and H. sativum. Sorption (collectively, adsorption and absorption) of the dimethyl compound, however, was significantly less than the di-n-propyl homolog.

Assuming that the dimethyl compound may actually be sorbed in amounts equivalent to or greater than the di-n-propyl homolog, it would be necessary to postulate that the derivative, in traceable form, must have been more weakly retained by the spore. If this were true, then, of the two compounds, relatively enormous amounts of the dimethyl derivative would be removed during filtration or by some other means. Since a 10 ml wash was usually used in the radiotracer work, this possibility was examined by measuring the radioactivity of unwashed spores. It was found that the methyl compound was more readily removed by washing, but the amount of di-n-propyl compound before washing far exceeded the amount of the dimethyl derivative.

Apparent sorption of the dimethyl compound would also be low if carbon disulfide or hydrogen sulfide resulted from the action of the biological system on the fungicide. No evidence of such compounds was obtained. The same sorption difference was apparent when spores were killed before exposure to the radioactive solutions of the derivatives. Furthermore, this difference in sorption was found after spores were exposed to the dithiocarbamates for 5 minutes, a treatment which resulted in only slight

inhibition of germination. It must be concluded then, that sorption of the less fungicidal di-n-propyl compound is indeed greater than that of the dimethyl homolog.

Whether or not the di-n-propyl compound is actually nontoxic remains to be evaluated. To verify the premise of inherent toxicity differences between the compounds, and analysis of relative concentrations within the protoplasm would be necessary. That is, distinction must be made between absorption and adsorption. Efforts to distinguish sorption by autoradiography of spore sections were unsuccessful. However, whether adequate resolution can be obtained with $S^{35}$ to distinguish relative concentrations in or on the spore wall and in the protoplasm, would require more extensive investigation. If it were necessary to differentiate concentrations at the spore wall-cytoplasm interface, the value of $S^{35}$ would then be questionable.

Evidence exists, however, that the di-n-propyl compound may adsorb strongly on the spore wall, and, therefore, not reach the site of action; greater adsorption of the di-n-propyl derivative on the plastic filters; and less desorption of this homolog than of the dimethyl compound by washing.

Even though the cytoplasmic membrane of the spore may be more permeable to the di-n-propyl compound by virtue of its greater lipoid solubility, it is nevertheless possible that many of the di-n-propyl molecules may be adsorbed by the spore wall, and, therefore, never come into contact with the lipoid membrane.

## SUMMARY

From the methods presented earlier (29), a procedure was modified to obtain reproducible measurements of the sorption of $S^{35}$ - labelled dithiocarbamates by fungus spores, based on an "infinitely thin" spore sample. Concurrent with the bioassay this radiotracer technique was used to evaluate the correlation between sorption and fungicidal activity of the dialkyldithiocarbamates, using H. sativum and M. fructicola.

In these tests sorption of the di-n-propyl derivative was consistently greater than the more fungicidal dimethyl homolog. Although the same sorption pattern occurred with M. fructicola, differences were less marked as were differences in fungicidal activity. Washing treated spores removed a greater proportion of the dimethyl than the di-n-propyl derivative. Spores which were heat-killed before exposure to solutions of the dithiocarbamate homologs sorbed the same relative amounts of the derivatives as nonheat-killed spores. With both compounds, sorption increased as duration of exposure increased from 5 minutes to 24 hours. Fungicidal activity of the dimethyl homolog also increased during the 24-hour exposure period. Sorption differences, evident at the end of the first 5 minutes of exposure, increased slightly during the remaining period. Under test conditions then, sorption of the dialkyldithiocarbamates was inversely correlated with fungicidal activity. Efforts to locate the site of concentration of these derivatives by autoradiography of spore sections were not successful.

Consequently, whether the di-n-propyl compound is nonfungicidal and absorbed in large amounts, or whether the homolog is so strongly adsorbed that fungicidal amounts do not permeate the spore, is not known. Relatively strong sorption of this compound on the plastic "Millipore" filter, its relatively low rate of desorption, and greater number of $CH_2$ groups are evidence for the latter possibility.

In investigations on the influence of carbon sources on susceptibility of H. sativum to the dimethyl compound, spores from media containing galactose, lactose, and starch, were most resistant. This was most pronounced at $2x10^{-4}$ molar concentration of the dimethyl derivative. In studies with glucose concentrations, marked differences in susceptibility were not observed. Both carbon source and concentration of glucose influenced sporulation and spore size.

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## AUTOBIOGRAPHY

I, George Donald Munger, was born in Wilmington, Delaware, May 21, 1923. My secondary school education was obtained at Claymont High School, Claymont, Delaware. My undergraduate education at the University of Delaware was interrupted by two and one-half years' service with the 10th Armored Division. In 1948, I received the Bachelor of Science degree, and, in 1949, the Master of Science degree from the University of Delaware. From 1949 to 1950, I was employed as a technical service representative by the duPont Company. While employed by Battelle Memorial Institute (1950-1954) I was a Research Engineer (1950-1952), and Principal Plant Pathologist (1952-1954). Since 1954, I have been employed as a research assistant in the Department of Botany and Plant Pathology, The Ohio State University.